DEVELOPMENT OF TEST PARADIGMS FOR OPERANT CONDITIONING OF WILD NORWAY RATS

SUSAN M. JOJOLA, USDA, APHIS, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA

PATRICK W. BURKE, USDA, APHIS, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA

GARY W. WITMER, USDA, APHIS, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA

DALE L. NOLTE, USDA, APHIS, Wildlife Services, National Wildlife Research Center, Fort Collins, CO, USA

Abstract: Many eradication efforts to remove rats (*Rattus* spp.) from islands have been successful. Eradications are expensive and labor-intensive which makes early detection of, and response to, reinvasion by rats critical. A better understanding of rat behavior could facilitate early detection and rapid response to intercept invaders, such as with trap placement and design, and toxic bait presentation and dispersal. This was a methods development study of test paradigms to operantly condition wild rats to run on an activity wheel and to press a lever for use in future behavior studies. Operant conditioning is the process of associating specific responses with specific reinforcers. The purpose of this study was to estimate the timeframe needed to operantly condition rats on wheels and levers, and to develop ideal test paradigms for conditioning these responses. Results indicated that wild Norway rats (*R. norvegicus*) need about 14 sessions, including adaptation, to reach a steady-state performance on an FR 2 schedule in wheel trials. Rats may need at least 21 sessions to adapt and shape a lever-press response, and 7-14 additional sessions to optimize the response on an FR 1 schedule. Individual variation in activity levels and learning rates was observed, suggesting a complexity to predicting the behavior of invading rats.

Key words: activity level, activity wheel, behavior, eradication, invader behavior, invasive species, lever press, Norway rat, operant conditioning, *Rattus norvegicus*, reinforcement rate

Proceedings of the 12th Wildlife Damage Management Conference (D.L. Nolte, W.M. Arjo, D.H. Stalman, Eds). 2007

INTRODUCTION

Rats (*Rattus* spp.) have been introduced onto over 80% of the world's islands or island chains (Atkinson 1985). Typically, rats have a significant negative impact on the abundance and distribution of native flora and fauna especially in cases where native species evolved in the absence of mammalian predators and have limited morphological, behavioral, and life history defenses against rats (Brown 1997). As a result, rats are implicated in 40-60% of recorded bird and reptile extinctions since 1600 (Groombridge 1992). Thus, removal of rats from islands is a key focus of resource managers. Many eradication efforts to remove rats from islands have been successful (e.g., Veitch and Clout 2002) and most often include systematic, intensive trapping and/or a blanket application of toxic baits (e.g., Parkes and Murphy 2003). Eradications are expensive and labor-intensive which makes early detection and response critical. Thus, a better understanding of rat behavior immediately after invasion is a key research priority for many resource managers.

Behavioral information (e.g., exploratory behavior, risk-taking, social dominance, resource time allocation, foraging) could facilitate more effective approaches to detection, trap placement and design, and/or toxicant bait dispersal and presentation, which could result in early interception of the invading rats. Operant conditioning is the process of training rats to associate that specific behaviors (e.g., lever pressing or wheel running) result in specific rewards, or reinforcers (e.g., food, water, access to a mate). A schedule of reinforcement is a prescription that states how and when discriminative stimuli and behavioral consequences will be presented (Morse 1966). The advantage to laboratory behavior studies using operant conditioning is that rats can be released into simulated island environments containing the operant chambers, which the rat has identified as its food source (or other conditioned response-reinforcer association), and data can be collected regarding a rat's preferences and priorities for food while other variables are manipulated in the simulated environment. Quantitative measures such as feeding frequency, duration, and amount can also be obtained. Information we hope to gain from future studies using behavioral instruments includes how rats exploit resources (e.g., food, water, shelter) in unfamiliar environments, the dispersal rate of rats in new environments, the trappability of invader rats, and the likelihood of invader rats to enter bait stations. This information would provide a better understanding of a rat's behavior in new environments and presumably facilitate a more accurate, targeted removal.

This study was a methods development study to determine the number of training sessions required to develop operant responses in wild Norway rats (*R. norvegicus*) based on specified schedules of reinforcement, and to develop ideal test paradigms for conditioning wheel-running or lever-press responses.

METHODS

Operant chambers (Med Associates, Inc., St. Albans, VT) were equipped with a house light, stimulus light, wheel or lever, pellet trough with head-entry detector, and pellet dispenser. Sucrose pellets served as reinforcers. Experimental contingencies were programmed using MED-PC programming language, and trials were recorded with a desktop PC using a Med Associates interface. Visual barriers were placed around operant chambers to minimize distractions. Trials were run in darkness between 7 am and 12 pm in the Animal Research Building of the National Wildlife Research Center, Fort Collins, CO. The room temperature was maintained at about 70-72º F with a 12 hrs on (12 pm to 12 am) – 12 hrs off (12 am to 12 pm) light cycle. Rats were housed individually in rack cages and given rat chow and water *ad libitum* while not on test. Prior to trials, *ad libitum* rat chow was slowly decreased over a period of about two weeks to achieve 80-85% of an individual's free-feeding body weight. This is a common practice when a hunger drive is necessary for lab studies. Rats were weighed daily and given chow rations following their session to maintain their decreased weight throughout the trial. During trials, individual rats were removed from their home cage, weighed, then placed in the operant chamber. The red house light within chambers signaled the beginning of a session. Infrared video cameras monitored the movements of rats during sessions. Upon termination of a session, house lights were turned off, no reinforcers were dispensed, and rats were returned to their home cage. Rats had one session each per day. Grid floors and catch pans of chambers were cleaned between rats when necessary.

First-generation offspring of locally (Fort Collins, CO) trapped wild Norway rats were used. Rats were experimentally naïve and randomly assigned to a group such that sex ratios were even within each group. Ten rats (five males, five females) were conditioned to run on an activity wheel (Wheel group), and 10 were conditioned to press levers (Lever group). Approximately 2 months later, rats in the Lever group were subjected to the wheel schedules of reinforcement without additional conditioning (Wheel Post-Lever group).

Examples of schedules of reinforcement include (from Pierce and Cheney 2004). Continuous Reinforcement (CRF), where every response required by the contingency is reinforced, and Intermittent Reinforcement, where some rather than all responses are reinforced. Ratio Schedules are a Fixed Ratio (FR), – reinforcers delivered after a fixed number of responses or a Variable Ratio (VR) – the number of required responses for reinforcement changes after each reinforcer is delivered. Interval Schedules are either Fixed Interval (FI) – a response is reinforced after a fixed amount of time passes or a Variable Interval (VI) responses are reinforced after a variable amount of time passes.

We used primarily FR schedules because we wanted rats to make a strong association between the required response and subsequent reinforcer. Thus, we incorporated a required head-entry (recorded by infrared beam) into schedules of reinforcement so that rats were required to consume a dispensed pellet (collect their reward) after each correct response before a subsequent pellet could be earned. By doing this, rats would also associate a flash of the stimulus light with delivery of the reinforcer. We did not investigate responses on different types of schedules (e.g., FI, VR, VI).

Training Sessions

Adaptation: All training sessions began with an adaptation period where rats were individually placed in chambers without access to the wheel or lever (up to 45 min/session). Adaptation served to familiarize rats with the enclosure and train them to associate a flash of the white stimulus light with the availability of a sucrose pellet. Pellets were hand-delivered by the observer via a push button while rats faced the pellet trough and could see pellets as they were dispensed. Pellets were repeatedly delivered while the rats were feeding to begin shaping the pellet-stimulus light association. Eventually, pellets were delivered while rats were not facing the trough. Rats were considered adapted when they could be drawn to the pellet trough by the stimulus light at least three times within a session. Inactive or sleeping rats were stimulated by the observer either by tapping on the chamber or reaching into the chamber and moving the rat.

Wheel Group: Wheel-running is intuitive for rats, so hand-shaping this response by the observer was not necessary. Following adaptation, all rats were put on a computer programmed FR 2 schedule of reinforcement for 12 days, then an FR 5 schedule for 6 days. Sessions lasted 30 min and were run 7 days/week in July and August 2006.

Lever Group: Hand-shaping a lever response by the observer followed adaptation of rats in the Lever group because pressing a lever is not as intuitive as running. The observer delivered reinforcers when a rat performed a posture or response that approached a lever-press. Rats graduated from hand-shaping when they pressed the lever (intentionally or inadvertently) several times within a session or came into frequent contact with the lever (e.g., touching but not pressing, nudging, biting, etc.) indicating an early association between the lever and pellet delivery. It was noted early on that various schedules would be needed to encourage activity by rats, so, not all rats were exposed to the same experimental contingencies. Following hand-shaping, rats were placed on FR 1, FR 3, and CRF (in this case, no head-entry required to earn a subsequent pellet) schedules. Some sessions consisted of access to a standard rolled lever, others to a retractable lever (RTR) that became inaccessible to rats after each correct response and until a head-entry was performed. Three relatively inactive rats were in 3-hr sessions; other sessions lasted 30 min (Table 1). Sessions were run 7 days/week in November and December 2006.

Table 1. Schedules of reinforcement and duration for rats in lever trials.

Rats (n)	1st schedule	Days	2nd schedule	Days
2	FR 1	9	FR 1 RTR	9
2	CRF	9	FR 1 RTR	9
3	CRF (3 HRS)	8	FR 1 RTR (3HRS)	8
1	CRF	12	FR 3	6
2	FR 1 RTR	9	none	

Wheel Post-Lever Group: Rats in this group were the same rats from the Lever group. It was expected that these rats might reach a steady-state performance sooner than rats in the Wheel group, due to their previous operant experience. About 2 months lapsed from when the rats completed lever trials to beginning wheel trials, without additional training. Rats in this group were on an FR 2 schedule for 12 days in February-March 2007. Sessions lasted 30 min and were run 4 days/week, rather than 7 days/week as in previous trials.

Steady-state was achieved when daily responses by rats did not change much from previous days while on a given schedule. Steady-states were determined by visual inspection of graphs depicting the average reinforcement rates [100-(number of reinforcers delivered ÷ number of responses) x 100)] per session for all rats within a group. Descriptive statistics were generated for the average number of responses, average number of reinforcers, and average reinforcement rate. The objectives of this study were to: 1. Determine the number of sessions required for rats to adapt to the operant chamber; 2. Determine the number of sessions required to hand-shape a lever-press response; and 3. Determine the number of sessions required to reach a steady-state performance on wheels and levers.

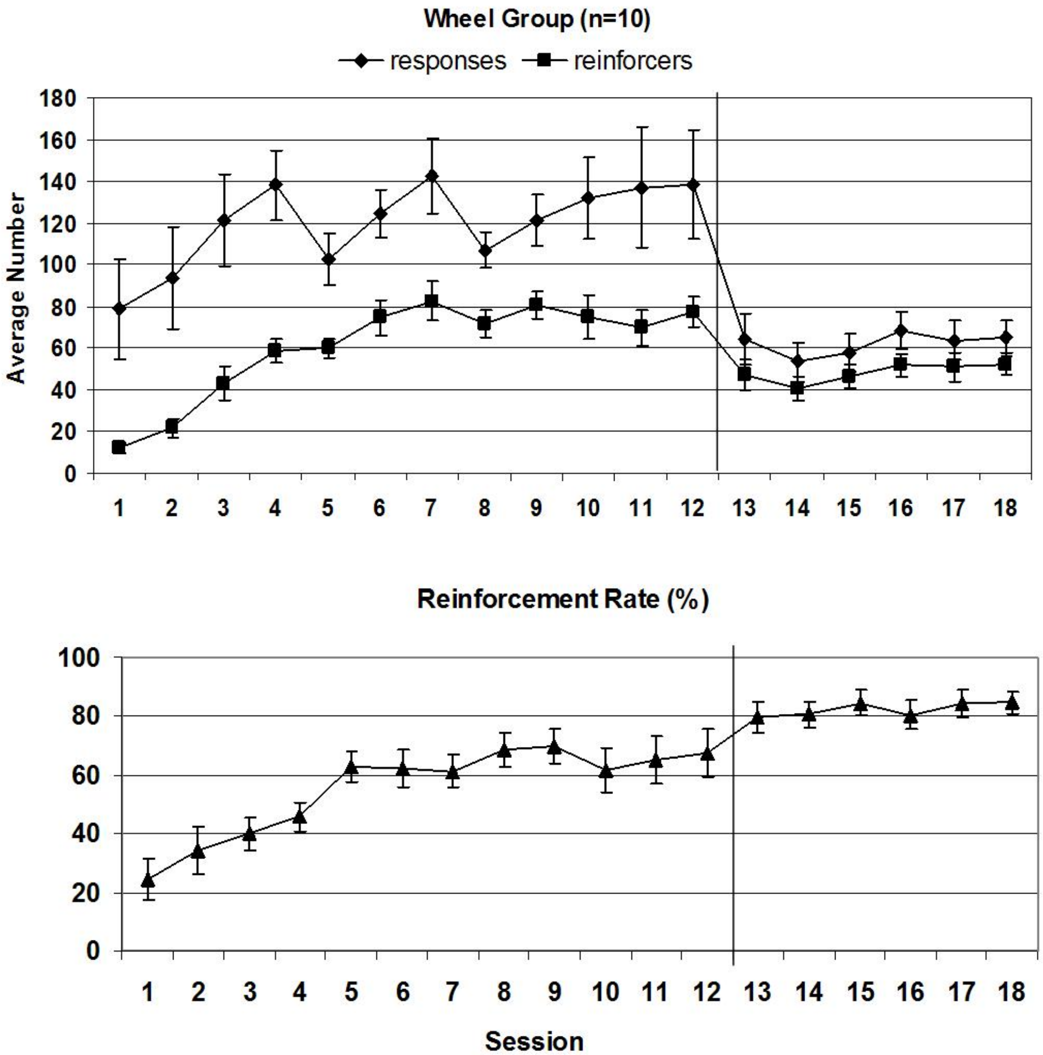

Figure 1. Values and rates of wheel sessions used to determine steady-state performance on two schedules of reinforcement. Rats seemed to reach steady-state by session 7 on their first schedule, and optimized their performance in sessions 8 and 9. Rats reached a steady-state and optimized on their second schedule by session 3.

RESULTS

Adaptation: Rats (n = 17) adapted to chambers and associated stimulus lights with dispensed pellets in 7 sessions, on average (range = 5-13). Three rats did not adapt after 7, 10, or 13 adaptation sessions. More time (i.e., 10 or 13 days) was given if rats were slightly active; less time (7 days) if they slept or were inactive. All rats were advanced to schedules of reinforcement regardless of their adaptation performance.

Wheel Group: Rats seemed to reach a steady-state by session 7 on an FR 2 schedule as indicated by average reinforcement rates (Figure 1). By session 7 there was not much change in the number of responses per reinforcer within the last 3 sessions. Sessions 8 and 9 showed a slight increase though not significantly, and subsequent session rates did not vary much from session 7. After session 8, average responses by rats became more variable.

This could indicate a sense of boredom or sucrose satiation. The rats optimized their performance in their 8th and 9th sessions, on average, meaning fewer excess responses were performed per reinforcer earned (the ratio of required responses to a single earned reinforcer was closer to 1:1). When advanced to an FR 5 schedule, a steady-state was reached by about the 3rd session (Figure 1). So, it took rats 3 sessions, on average, to learn that they must respond 2.5 times more than previously for the same reinforcement. Rats also optimized their performance on the FR 5 schedule by session 3. Responses of rats on the FR 5 schedule were less variable than toward the end of the FR 2 schedule. This may further indicate that rats may have become disinterested in sessions toward the latter part of the FR 2 schedule.

Lever Group: Most (n = 6) rats were hand-shaped for a lever-press response within 7 days. The other four rats (including two that did not meet adaptation criteria) did not meet the criteria for “hand-shaped” but were advanced to schedules of reinforcement anyway (Table 1). Most rats (n = 9) were relatively inactive on their respective schedules regardless of lever type or session duration. Specifically, 1,096 responses were made by all rats during their first schedule, 91% of these were by a single rat (Figure 2). Sample sizes were too small for each schedule and too few responses were recorded to determine the number of sessions to achieve steady-state in Lever trials. More time may be necessary in the hand-shaping stage and other motivational techniques may be needed to shape and condition lever-pressing. Despite inactivity in lever trials, the same rats were more active in wheel post-lever trials.

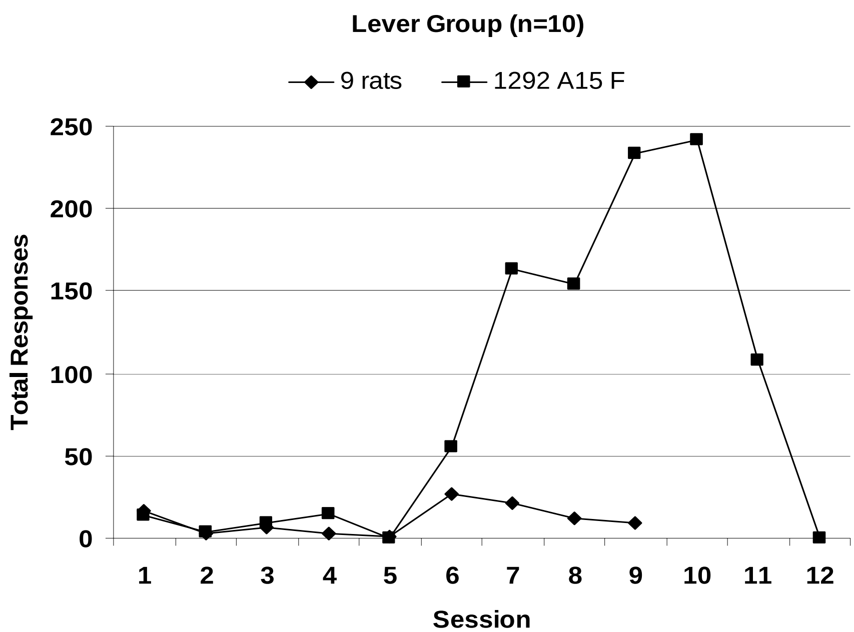

Figure 2. Number of responses by rat 1292 A15 F and by all other rats on their first schedule of reinforcement in Lever trials. Rat 1292 A15 F was the most active, having 91% of all responses made on first schedules.

Wheel Post-Lever Group: On average, rats reached a steady-state on the FR 2 schedule by their 7th session (Figure 3). It was expected that rats might perform better than rats in the Wheel group due to their previous experience on levers. Instead, the rats were as active as rats in the Wheel group, but earned fewer pellets, which resulted in lower reinforcement rates (Figure 4). This decrease in reinforcement rate may be due to 1) lack of interest in pellets by this group, 2) preference for physical or mental stimulation over pellets, 3) time since adaptation (3 months), 4) difference in number of sessions run per week, and/or 5) instrument error. Sources of instrument error were further investigated and are described below.

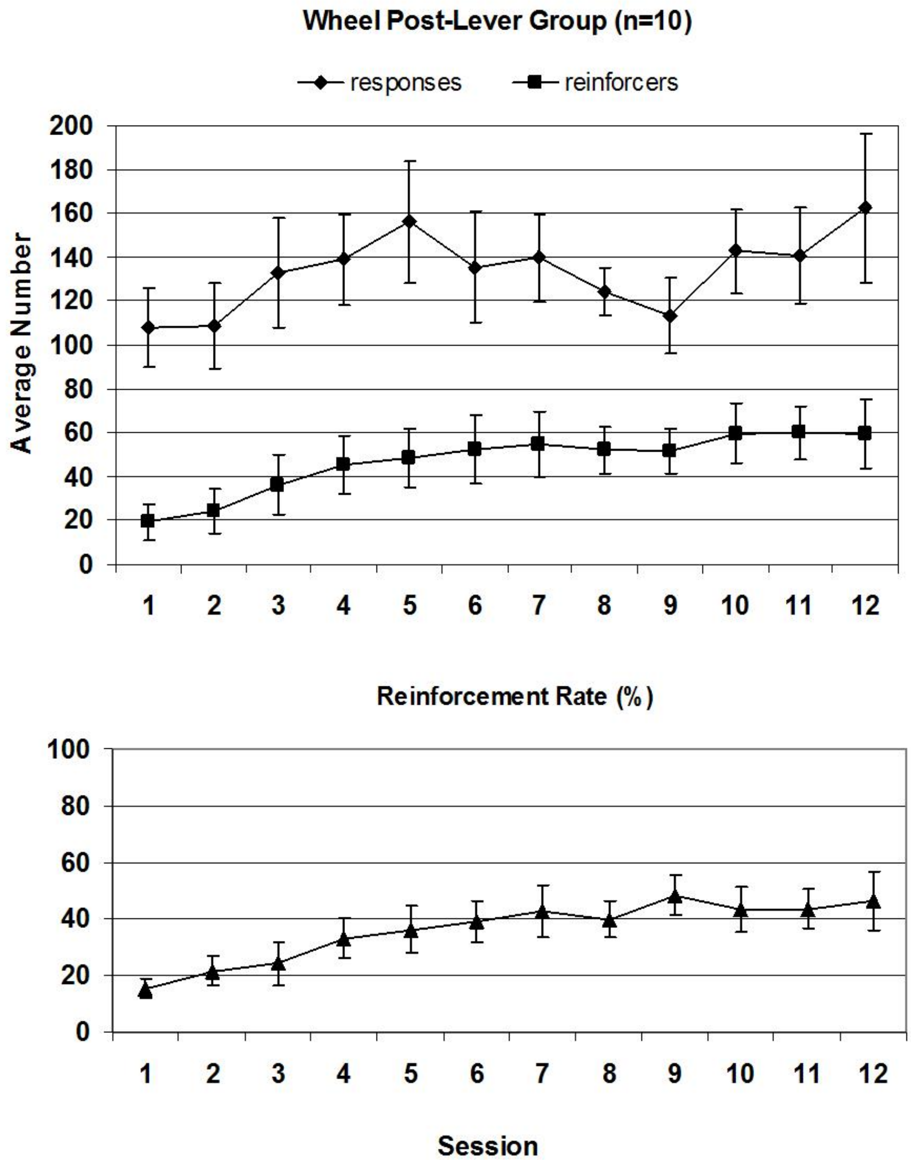

Figure 3. Values and rates of wheel post-lever group sessions used to determine steady-state.

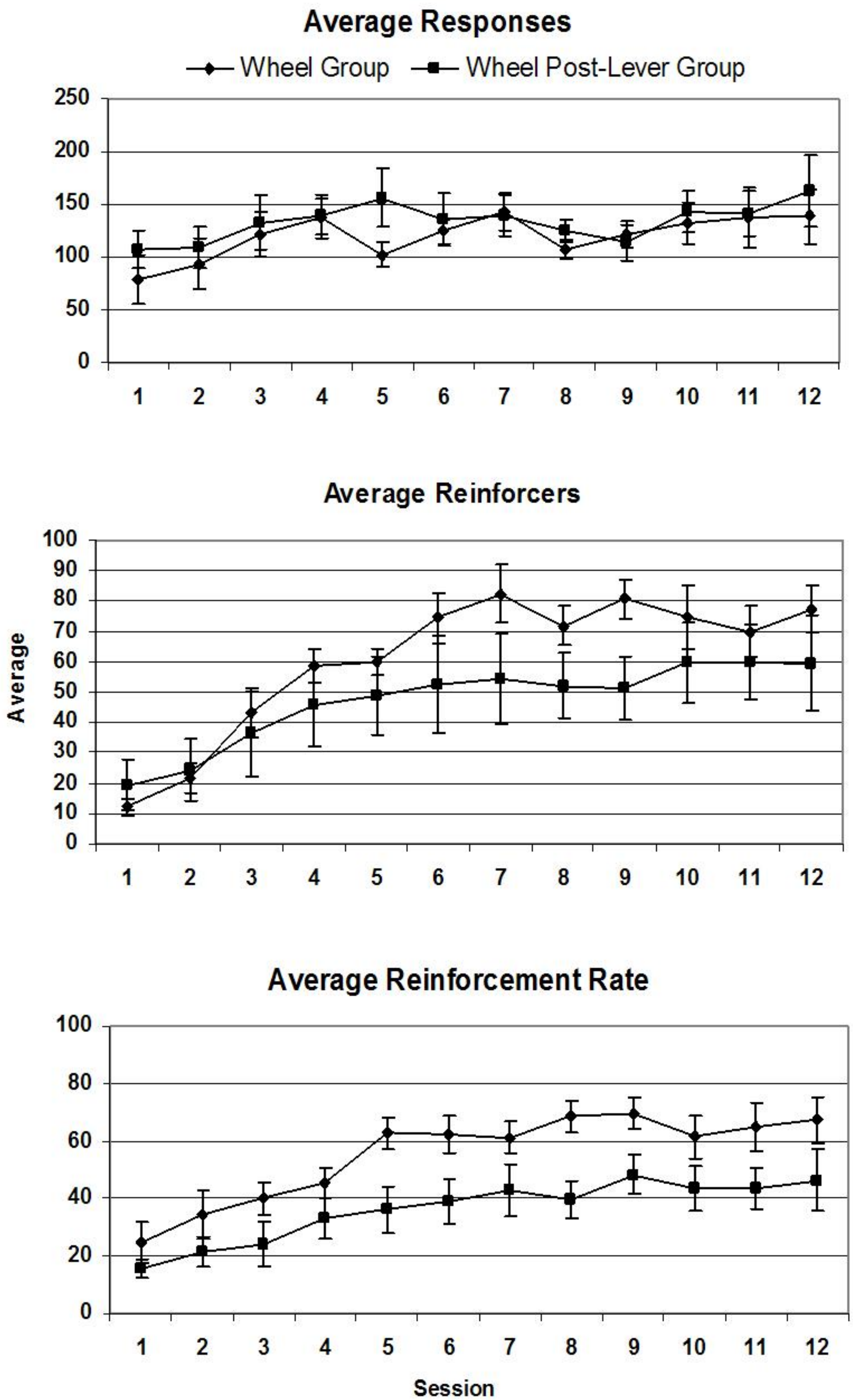

Figure 4. Visual comparison of the Wheel Group and Wheel Post-Lever Group trials.

The discrepancy in reinforcement rates between the Wheel and Wheel Post-Lever groups seems to be attributable to instrument error of four additional operant chambers used for the wheel post-lever trials. On a per session basis across all trials, pellet troughs in chambers 1 and 2 averaged catch rates, meaning troughs successfully held dispensed pellets, of $\geq$ 99.6% and 98.4%, respectively (Table 2). Likewise, on a per session basis across lever and wheel post-lever trials, pellet troughs in chambers 3, 4, 5, and 6 averaged catch rates of $\geq$ 98.7%, 88.6%, 90%, and 96.9%, respectively. Chambers 4, 5, and 6 were not as consistent in catching dispensed pellets as the other 3 chambers. This variability in catching pellets is noticeable in the different reinforcement rates (Figure 4). So, it was not the case that rats learned less efficiently in the wheel post-lever trial. Rather, it was because of instrument error, which caused the responses and reinforcers to be skewed.

Table 2. Average catch rates ($\pm$SE) of pellet troughs per session during three trials based on pellets that bounced out of the trough, through the grid floor, and onto the catch pan where they were unavailable to rats.

Chamber	Average Catch Rate per Session[a]	SE	Sessions (*n*)
Wheel Group			
1	99.8	0.07	78
2	99.3	0.14	102
Lever Group			
1	100.0	0.00	18
2	98.7	1.00	13
3	99.3	0.37	29
4	88.6	11.08	9
5	90.0	10.00	5
6	96.9	3.02	11
Wheel Post-Lever Group			
1	99.6	0.15	27
2	98.4	0.58	21
3	98.7	0.64	21
4	99.4	0.30	15
5	98.9	0.36	29
6	98.7	0.68	22

[a] Does not include manually-delivered pellets

Instrument Error: During trials, we noted two types of instrument errors. On several occasions pellets bounced out of the pellet trough, through the grid floor, and into the catch pan becoming unavailable to rats. If rats did not perform a head-entry into the trough (which also resets the stimulus light), a subsequent correct response was not reinforced. Often, rats could see that a pellet was not in the trough and would continue to respond correctly without being reinforced accordingly. Secondly, there were occasional "misfires" of the pellet dispenser, meaning the pellet magazine advanced, but a pellet was not dispensed. Both unavailable and misfired pellets potentially could bloat a rat's responses while reinforcers remained constant, which would drive down the reinforcement rate. If the observer witnessed an unavailable or misfired pellet, a pellet was delivered manually via the computer. However, not all of these occasions were observed.

To obtain an indication of catch rates for each chamber's pellet trough, after trials we examined raw data where rats performed at least one response in a session. Catch rates were calculated for each session and then these rates were averaged for each chamber in each of the three trials (Table 2). Chamber 4 had the highest variability in catch rate: 88.6%, on average, per session during lever trials, and 99.4% , on average, in wheel post-lever trials. So, rats placed in chamber 4 in lever trials were not reinforced accurately according to their schedule, which affected overall learning and reinforcement rate. Although most catch rates seem high (upper 90's), one unavailable or misfired pellet modifies the schedule of reinforcement which is the critical training tool for operant conditioning.

To increase the catch rate of pellet troughs, the manufacturer recommended adjusting the rubber tube that pellets travel down from the dispenser to the trough, and bending the deflector plate above the food trough that pellets hit before dropping into the trough so that just enough space is available for a pellet to fit through. We have not yet conducted quality control trials using these modifications to determine if catch rates improve. Depending on the objectives of a study, another option to minimize the effects of a poor catch rate is to run schedules that do not require a head-entry before earning subsequent reinforcers. This would allow the rat to earn a pellet for every contingent response. If a pellet bounces to the floor, only one response is not reinforced rather than potentially several before a head-entry is performed.

CONCLUSIONS

The purpose of this study was to estimate the timeframe needed to operantly condition wild Norway rats on wheels and levers for use in behavioral studies, and to develop ideal test paradigms for conditioning these responses. An FR 2 schedule with required head-entry seemed ideal for conditioning a wheel-running response. Under this paradigm, approximately 14 days are required for rats to adapt to and reach steady-state (this timeframe does not include decreasing free-feeding body weight of rats). Approximately doubling the responses of an FR schedule will take only a few days more to achieve steady-state. The catch rate of troughs is imperative to achieving valid results; quality control measures of pellet dispensers should precede each trial.

Lever-pressing was more difficult for rats to learn. Various techniques may need to be implemented to shape and condition a lever-press response in wild rats, such as decreasing session duration, placement of levers adjacent to pellet troughs (rather than opposite, as in our trials), and alternating lever trials with wheel trials or other more intuitive response. It is likely that rats will require about 21 sessions to adapt to chambers and hand-shape a lever-press response. An additional 7-14 sessions may be required to reach steady-state on an FR 1 or CRF schedule.

Rats from the Lever group retained their adaptation experience after 3 months and readily applied it in wheel post-lever trials. A steady-state was reached in wheel trials within 7 sessions by Wheel Post-Lever group rats, which is the same amount of time it took rats in the Wheel group to reach steady-state. We did not run trials to determine if the reciprocal were true: if rats previously trained on wheels readily apply their adaptation experience to lever trials in the same amount of time.

An important aspect of behavior was observed during this study. Namely, the activity level between individual rats varied significantly. Some rats could not be motivated to investigate the chamber or develop associations regardless of hunger or training techniques while others developed associations quickly and responded frequently and consistently. Individual activity levels may play a significant role in rats' exploratory behavior, which influences invader behavior. It may be feasible that rats with higher activity levels (have a high number of responses or learn associations quickly) may be more likely to successfully invade islands. Once on the island, it would be beneficial to know what these rats do next. That is we want to better understand their invader behavior. Do they immediately disperse inland for a distance, or do they loaf near the invasion site for a few days? Which resources do they initially exploit (e.g., food, fresh water, cover, conspecific odors)? Are active rats more or less likely to enter traps or bait stations used in current control efforts? Is it likely that less active rats are missed by eradication efforts? These are questions that can be investigated in behavioral studies using the test paradigms described in this study, and which we intend to investigate in future studies.

ACKNOWLEDGEMENTS

The following people assisted in study design or ideas, data collection, data input and manipulation, or generating a poster of results: Katelyn Goodwin, Rachael Piergross, Rodger Thompson, Matt McCollum, and Carl Cheney.

LITERATURE CITED

ATKINSON, I.A.E. 1985. The spread of commensal species of *Rattus* to oceanic islands and their effects on island avifaunas. Pages 35-81 *in* P.J. Moors, editor: Conservation of island birds. International Council of Bird Preservation Technical Publication 3.

BROWN, K.P. 1997. Predation at nests of two New Zealand endemic passerines; implications for bird community restoration. Pacific Conservation Biology 3:91-98.

GROOMBRIDGE, B. 1992. World Conservation Monitoring Centre, British Museum (Natural History), International Union for Conservation of Nature and Natural Resources. Global biodiversity: status of the earth's living resources: a report. Chapman and Hall, London, UK.

MORSE, W.H. 1966. Intermittent reinforcement. Pages 52-108 *in* W.K. Honig, editor. Operant behavior: Areas of research and application Appleton-Century-Croftsl, New York, NY, USA.

PARKES, J., AND E. MURPHY. 2003. Management of introduced mammals in New Zealand. New Zealand Journal of Zoology 30:335-359.

PIERCE, W.D., AND C.D. CHENEY. 2004. Behavior analysis and learning. Lawrence Erlbaum Associates, Inc., New Jersey, USA.

VEITCH, C.R., AND M.N. CLOUT. 2002. Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.